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NOTES

Effect of Temperature on Phagocytosis by Human Polymorphonuclear Neutrophils

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Monolayers of human peripheral blood polymorphonuclear neutrophils and ^{14}C-labeled *Staphylococcus aureus* were incubated at various temperatures. The rate and extent of phagocytosis was unchanged between 33 and 41 C but was depressed above and below this range.

Phagocytosis of bacteria by polymorphonuclear neutrophils (PMN) is an active, energy-requiring process, and thus would be expected to be temperature dependent. Since elevated body temperature is a nearly universal manifestation of infection, we examined the effect of temperature on phagocytosis of *Staphylococcus aureus* by human PMN with special attention to temperatures in the "fever" range. The system used employed PMN adherent to glass and a high multiplicity of bacteria to phagocytes. Ingestion of bacteria in such a system involves active motility, chemotaxis, and ingestion by the phagocytes. In contrast, a tumbling liquid system (the standard differential centrifugation assay [3]) is dependent only on random contact between bacteria and neutrophils. Both phagocytic rate and capacity were assayed by

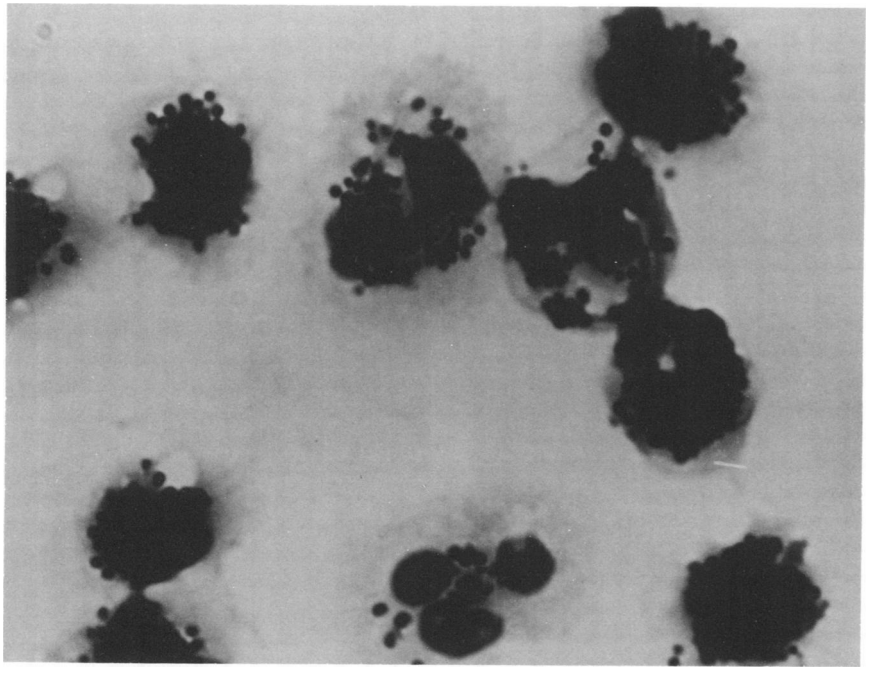

FIG. 1. *Photomicrograph of monolayer preparation incubated with staphylococci at 37 C for 30 min as described in the text. All bacteria in this field are cell associated and most appear to be intracellular. (Original magnification, ×1,000).*

utilizing excess labeled bacteria (100 bacteria per PMN) and sampling at frequent intervals (4).

^{14}C-labeled *S. aureus* were prepared by incubation at 37 C for 18 h in Trypticase soy broth containing 100 μCi of ^{14}C-labeled amino acid nixture per ml (New England Nuclear Corp.). The labeled bacteria were washed three times in saline, resuspended in the original volume, and killed by boiling for 30 min. Human PMN monolayers were prepared by incubating 0.3 ml of fresh blood from normal donors in 1.5-cm diameter glass micro-petri dishes at 37 C for 30 min. The clot was then carefully washed off with 37 C saline and 0.15 ml of Hanks balanced salt solution with 10% autologous serum was added to the dish. Such monolayers consist of about 2×10^6 phagocyte cells, 90 to 95% of which are PMN and 5 to 10% monocytes (7). The monolayers were equilibrated for 5 min in an agitated water bath (accurate to $\pm$ 1/4 C) at either 24, 33, 35, 37, 39, 41, 43, or 45 C, and 0.5 ml of the ^{14}C-labeled bacterial suspension (about 2×10^8 organisms) was then added to the dishes. At 0, 10, 30, 60, and 90 min nonphagocytized bacteria were removed by washing four times with saline at 24 C. Organisms that were adherent to the dishes were prepared for liquid scintillation counting by solubilization with 0.3 ml of Protosol (New England Nuclear Corp.) for 5 min, suspended in counting solution, and counted in a Beckman LS 250 scintillation counter. Microscopic examination of stained monolayer preparations showed that after washing nearly all bacterial were cell associated and appeared to have been ingested by PMN as determined by bacteria in the proper plane of focus and vacuolization (Fig. 1). Controls were done with dishes without PMN monolayers. All experiments were done in quadruplicate and PMN from the same donor on the same day were used to study phagocytosis at 37 C and at another temperature.

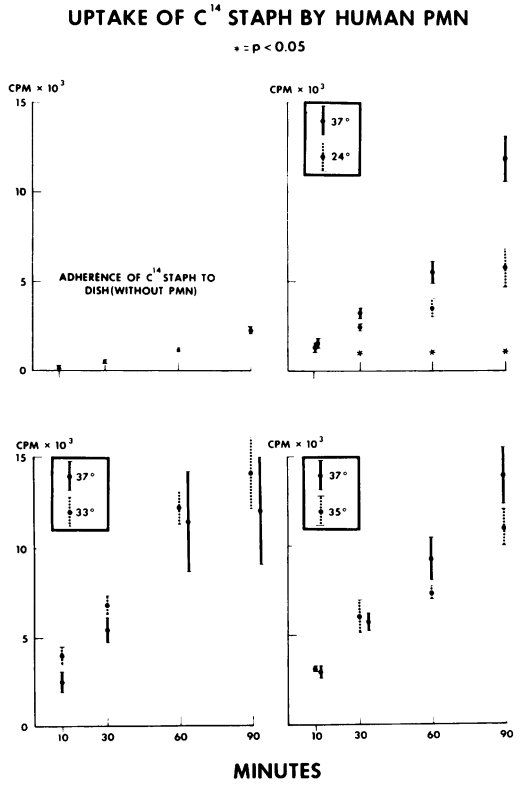

FIG. 2. *^{14}C-labeled Staphylococcus aureus were incubated with PMN monolayers or blank dishes. Points indicate mean cell associated counts ± standard error of the mean (n = 4 for each point). Each graph represents paired studies done with PMN from the same donor. *, Significant difference (P < 0.05, student's t test) between means of values at 37 C compared to 24, 33, or 35 C. The slope indicates rate of ingestion. There was slight adherence of staph to blank dishes. Uptake of staph was significantly impaired at 24 C but was equivalent to that seen at 37, at 33, and 35 C.*

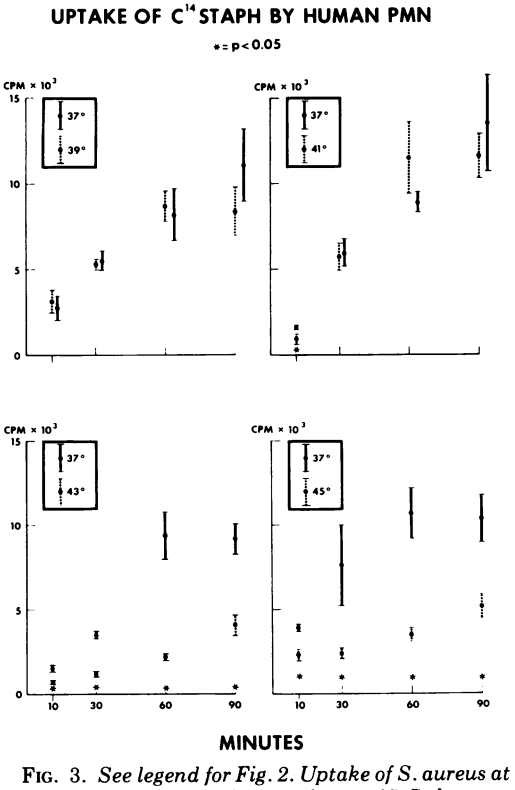

FIG. 3. *See legend for Fig. 2. Uptake of S. aureus at 39 and 41 C was equivalent to that at 37 C, but was markedly impaired at 43 and 45 C.*

Results are shown in Fig. 2 and 3. There were many more bacteria adherent to dishes with PMN monolayers than to blank dishes. Uptake of staphylococci by PMN was equivalent at 33, 35, 37, 39, and 41 C. Phagocytosis was significantly depressed at 24, 43, and 45 C when compared to 37 C.

In 1942 Ellingson and Clark (2) counted numbers of ingested bacteria per leukocyte after 15 min of incubation at various temperatures. They found 38.4 bacteria per cell at 37 C; 50.0 at 38 C; 53.3 at 39 C; 52.8 at 40 C; 46.4 at 41 C; and 42.7 at 42 C. No range or standard error of the mean was given. Bryant et al. (1) and Nahas et al. (5) found increased rates of locomotion at higher temperatures. However, Phelps and Stanislaw (6) found no significant change in PMN motility between 98 and 105 F.

The elevated temperature that clearly inhibited PMN phagocytic function (43 C) is higher than the central body temperature reached in most disease states, but this temperature may be reached in an extremity that is being treated with "hot soaks" for soft tissue infection.

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